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Life cycle and characterization of *Neoglyphe locellus* (Kossack, 1910) (Digenea: Plagiorchiidae) from Bulgaria

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Summary

After experimental reproduction of *Neoglyphe locellus* (Kossack, 1910) life cycle was found that *Planorbarius corneus* (L., 1758) snail can be a second intermediate host for this species. All stages of parasite's development were described, including argentophylic and chromosome structures. A comparative analysis of published data concerning *N. locellus* was performed. The degree of variation of some taxonomic important features in populations from different geographic areas was followed up.

Key words: *Neoglyphe locellus* (Kossack, 1910); life cycle; chaetotaxy; karyotype

Introduction

Opisthioglyphe locellus (Kossack, 1910) was described originally from the small intestines of *Sorex fodicis*. Schaldybin (1953) divided the genus *Opisthioglyphe* into two subgenera - *Opisthioglyphe*, which includes amphibian parasites, and *Neoglyphe,* which includes mammalian parasites. Yamaguti (1958) declared *Neoglyphe* Schaldybin, 1953, an independent genus and placed it in the subfamily Omphalometrinae Looss, 1899, of the family Plagiorchiidae (Lüche, 1901).

The life cycle of *O. locellus* was first studied by Macey and Moore (1958) in America. Bock (1982) described the life cycle of the species based on material from Europe (Germany) however *Planorbarius corneus* (L., 1758) was not recognized as a second intermediate host. In Bulgaria adult *N. locellus* was found in intestine of *Crocidura leucodon, C. suaveolens, Neomys anomalus, Sorex araneus* and *S. minutus* by Genov and Dimitrova (1966), Genov (1979, 1984), Prokopič and Genov (1974), but its life cycle has not been studied experimentally.

The cercarial chaetotaxy of *N. locellus* have been studied by Bock (1983). No data about karyological investigations of *N. locellus*.

The objective of this study was to provide full characterrization of *N. locellus* from Bulgaria, using biological, morphological and cytogenetical methods.

Material and Methods

A total of 726 snails of the species *P. corneus* for natural invasion were studied. They were collected in autumn (October) along the banks of the Danube. Thirty-two of them (4.40 %) were infected with *Xiphidiocercaria armata,* belonging to the *Plagiorchis*-group, according to types of Grabda-Kazubska (1971). After sectioning, it was found that 325 snails (44.77 %) contained metacercariae of *N. locellus* localized in hepatopancreas, pericardial area and muscles. Five hamsters (*Mesocricetus auratus*) were fed individually with tissues containing metacercariae of *N. locellus.* Seven days post infection, 390 mature specimens were obtained and 70 were morphologically analyzed after fixation and staining used by Radev *et al.* (1999). Cultures from 250 eggs spontaneously laid by the mature parasite specimens were incubated for 80 days at 15 – 18° C and used to study embryogenesis. Thirty daughter sporocysts, 70 cercariae and 90 metacercariae of different localization in the tissues of the naturally infected *P. corneus* were morphologically studied.

Twenty five cercariae impregnated according to Combes *et al.* (1976) were used to study the chaetotaxy. The topography of the surface tegumentary structures is described according to nomenclature of Bayssade-Dufour (1979), with some modifications, which concern the head area, suggested by Grabda-Kazubska and Kiseliene (1989).

Daughter sporocysts from 5 snails were used in the karyotype study. Twenty metaphase cells were karyometric analyzed statistically. Slides were prepared according to Mutafova *et al.* (2001).

The optical microscope "Zeiss Opton" with camera lucida, videomat and automatic photo-camera was used for microscopic examinations, measurements, photographs and drawings. All metric data are given in micrometres.

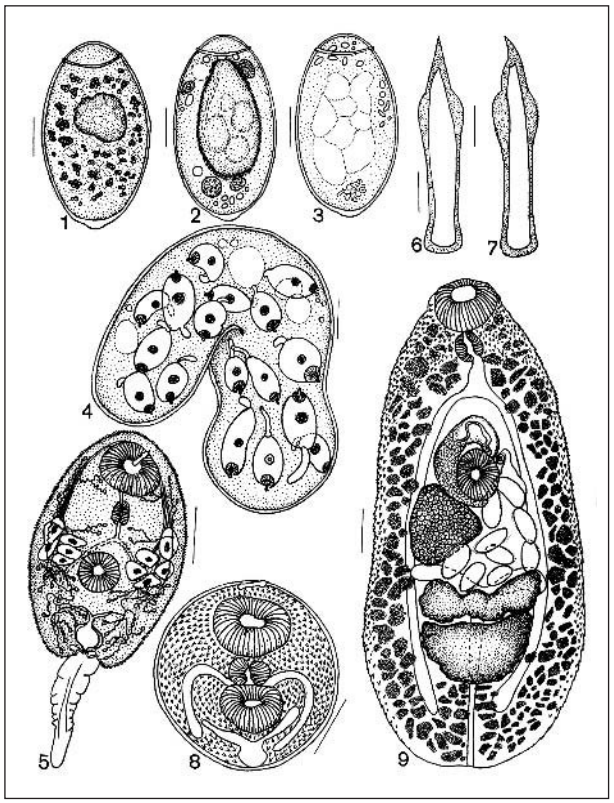
Results

I. Morphology and life cycle

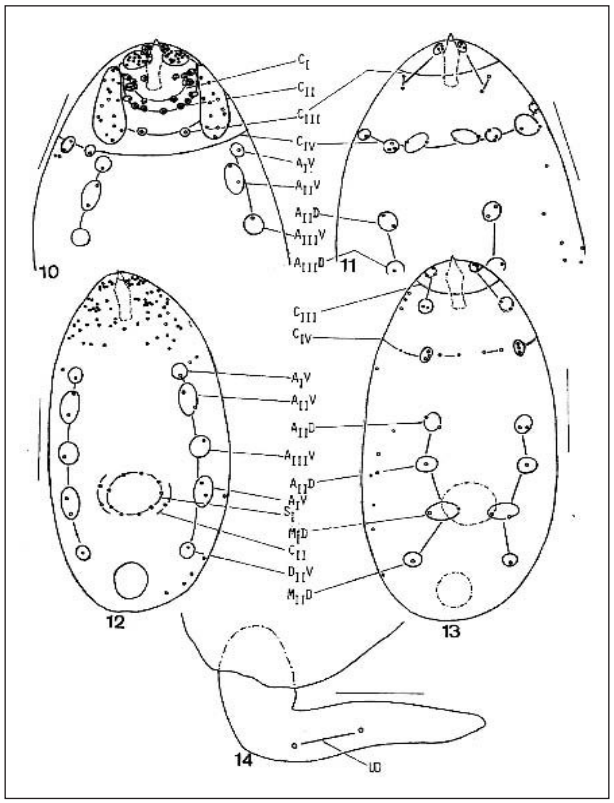
Eggs and miracidia. Spontaneously laid by adult trematodes eggs (Fig. 1) are elliptical to oval, operculate, yellow to light brown, unembryonated 45.5 – 52.78 (47.86 ± 3.816) in length and 26.4 – 35.2 (27.35 ± 3.245) in width. The operculum base measured 13.2 – 21.12 (16.72 ± 4.033) and 3.16 – 3.69 (3.45 ± 0.271) in height.

Daughter sporocysts isolated from the digestive gland of snail are sausage-like (Fig 4), usually U-shape and measured 360.93 – 720.27 (565.480 ± 125.422) in length and 120.4 – 250.32 (192.90 ± 39.833) in width. They contain germinative balls and 3 to 5 cercariae.

Cercariae. Living spontaneously emerged *Xiphidiocercaria armata* had a spinose body 228.35 – 371.03 (295.63 ± 35.64) long and 111.23 – 130.32 (124.70 ± 5.83) width (Fig. 5). The tail is without finfolds and spines 62.91 – 265.34 (143.03 ± 54.46) long and 19.35 – 47.13 (30.74 ± 8.17) wide. The stylet is 28.76 – 32.75 (29.98 ± 0.98) in length and 7.5 – 8.2 (8.07 ± 0.3) in width (Figs. 6, 7). Oral sucker is subterminal, elliptical to oval 42.44 – 70.70



Figs 1 – 9. *Neoglyphe locellus* (Kossack, 1910) - developmental stages: 1 – Egg at the moment of lay. Scale bar 10 µm; 2 – Egg after 12 days of incubation. Scale bar 10 µm; 3 – Egg containing unmovibal embryo. Scale bar 10 µm; 4 – Sporocyst. Scale bar 50 µm; 5 – Cercaria. Scale bar 50 µm; 6 – Stylet - dorsoventral view. Scale bar 10 µm; 7 – Stylet - lateral view. Scale bar 10 µm; 8 – Metacercaria. Scale bar 50 µm; 9 – Adult. Scale bar 50 µm



Figs 10 – 14. *Neoglyphe locellus* (Kossack, 1910) – Argentophilic structures: 10 – Papillae on the cephalic region – ventral view. Scale bar 30 µm; 11 – Papillae on the cephalic region – dorsal view. Scale bar 30 µm; 12 – Body papillae – ventral view. Scale bar 30 µm; 13 – Body papillae – dorsal view. Scale bar 30 µm; 14 – Tail papillae. Scale bar 30 µm

On the 12th day after egg lying, the miracidium has completed its development and showed active movements (Fig. 2). It is located near to the operculum and its dimensions are 42.84 in length, 22.70 in width. The miracidium does not leave the egg. Its mobility decreases from the 62nd to the 72nd day and after the 79th day it is immobile (Fig. 3).

(52.28 ± 7.05) in length and 47.79 – 70.70 (53.37 ± 6.24) in width. Ventral sucker is oval just after or on the middle of the body 34.95 – 53.50 (41.60 ± 5.57) in length, 35.45 – 48.97 (41.64 ± 3.87) in width. Oral to ventral sucker ratio is 1.4. Prepharynx is distinct, 5.24 long. Pharynx is oval well developed 16.8 – 21.17 (17.35 ± 3.891) in diameter.

The flame-cell formula is 2 [(3+3+3) + (3+3+3)]. Main excretory ducts lead into the lateral branches of the Y-shaped excretory bladder situated at posterior end of the body. Excretory pore is situated subterminal at the posterior body end. The penetration gland cells are located in two groups, symmetrically arranged beside and just anterior to the ventral sucker. Each group contains five to six gland cells and gland duct with openings at the base of the stylet. Cystogenous gland cells are spread all over the body. Cercariae survive for up to 20 – 40 h at 18°C. They have negative geotaxis and actively seek the second intermediate host. The infected snail host shedding cercariae during the twenty-four-hour period but most active process was observed in the morning between 06:00 and 08:00 am.

Metacercariae. Fully developed metacercariae isolated from naturally infected *P. corneus* are elliptical to oval 136.5 – 163.3 (152.43 ± 10.117) long and 95.2 – 118.4

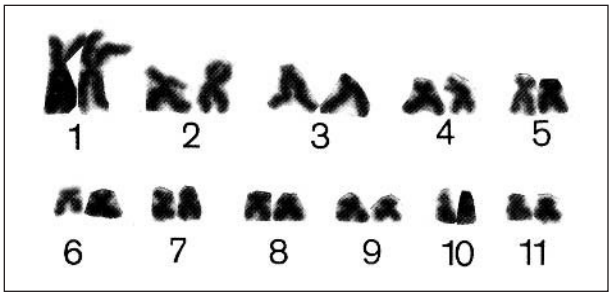


Fig. 15. *Neoglyphe locellus* (Kossack, 1910) karyotype. Scale bar 10 μm.

(116.8 ± 8.7) wide (Fig. 8). Metacercariae are localized in the hepatopancreas, pericardial area and muscle tissues of the snail hosts. No morphological differences were found in metacercariae isolated from different sites.

Adults. Seven days old experimentally obtained specimens are with heartshape to elliptical, dorsoventrally flattened, spinose body, 456.00 – 648.00 (565.00 ± 49.562) long and 240.00 – 300.00 (276.40 ± 16.320) wide (Fig. 9). Body spines are 2.00 – 3.00 long distinguished regularly on the body surface. Oral sucker is well developed, with elliptical to oval shape, subterminal 59.35 – 79.24 (68.15 ± 7.32) in length and 87.84 – 109.8 (97.49 ± 9.00) in width. Praepharynx present. Pharynx is well developed, elliptical, just post oral sucker 23.96 – 47.134 (34.52 ± 9.17) long. The ratio of oral sucker to pharynx lengths is 1.97. Oesophagus is short, intestinal bifurcation is on the level between the first and second fourth of the body. Intestine ends are blindly near to the posterior body end. Ventral sucker is oval situated before middle of the body with diameter 49.41 – 65.88 (61.12 ± 4.26). Oral to ventral sucker length ratio is 1.11. Ovarium is irregular in shape and located outside of the medial body line, post ventral sucker. Vitelline follicles are irregular in shape and different in size. Vitelline fields are symmetrical, extraintestinal, beginning just

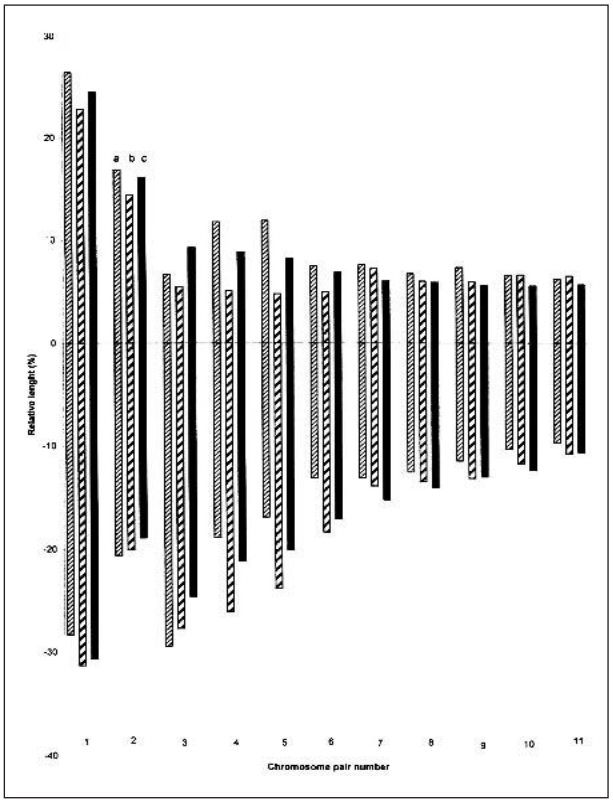


Fig. 16. Idiogram of chromosomes: a – *Neoglyphe locellus* (constructed on the data in Table 1), b – *Opisthioglyphe ranae*; c – *Plagiorchis macullosus* (by Mutafova, 1994)

post oral sucker, reaching to the posterior body end, curving around the end of intestinal branch and ending just post posterior testis. Cirrus is well developed, long 117.12 – 153.72 (140.60 ± 10.68), post ventral sucker before ovarium, outside of the medial body line, U-shaped around the ventral sucker margin. Body to cirrus pouch length ratio is 4.01. Male genital atrium is situated post intestinal bifurcation, before ventral sucker, outside of medial body line. Testes are in posterior half of the body, tandem with rounded surface. Anterior testis is 42.09 – 64.05 (53.34 ± 5.792) long and 109.8 – 164.7 (140.501 ± 18.26) wide. Posterior testis is 42.07 – 76.35 (57.27 ± 13.39) long and 81.04 – 160.04 (150.70 ± 13.64) wide. Uterus is short, between anterior testis and ventral sucker, containing about 10 unembryonated yellowbrown eggs. Metraterm is around ventral sucker opposite of the cirrus pouch ending with female genital atrium.

II. Argentophilic structures of N. locellus cercariae
The tegumentar papillae are situated as follow:

1. Cephalic region: (Figs. 10, 11)
C_I=1C_IV, 5C_IL, 1$C_I$$D_1$, 1$C_I$$D_2$
C_{II}=1$C_{II}V_0$, 1$C_{II}V_1$, 1$C_{II}V_2$, 1C_{II}L, 2C_{II}D
C_{III}=1$C_{III}V_1$, 15-18$C_{III}V_2$+$C_{III}L_1$, 14-22$C_{III}D_1$,

$3+2C_{III}D_2$
C_{IV}=0-1$C_{IV}V_1$, 0-2$C_{IV}V_2$, 1-2$C_{IV}L_1$, 0-2$C_{IV}L_2$, $3C_{IV}D_1$, $2C_{IV}D_2$

2. Body papillae: (Figs. 12, 13)
a) ventral papillae
$1A_{I}V$, $2A_{II}V$, $1A_{III}V$
$2M_{I}V$
$1P_{II}V$

b) dorsal papillae
$2A_{II}D$, $1A_{III}D$
$2M_{I}D$
$1P_{II}D$

c) acetabular papillae
$9S_{I}$, 0-$3S_{II}$
d) lateral papillae
about 20 papillae on each lateral body field.

3. Tail papillae (Fig. 14)
The papillae 2UD are in longitudinal line on the middle of the tail. Most of the papillae have constant number and location. The papillae $1C_{I}V$ are invaginated. Variations were obtained mainly in $P_{II}D$ and S_{II} papillae.
Acetabular papillae on the circle S_{II} in most of the investigated cercariae were not obtained. The papillae $1P_{II}D$ in some of investigated cercariae absent. The openings of the penetration gland cells were impregnated together with $C_{III}D_1$ papillae. They were irregular in shape and larger from the papillae.

centromere localization, the 1st, 2nd and 5th chromosome pairs are typically metacentric, the 3rd is subtelocentric, and 7 pairs (4th, 6th to 11th) have median - submedian classification.

Discussion

Our experimental studies showed that *P. corneus* is the first and can be a second intermediate host for *N. locellus*. According to Bock (1982) second intermediate host for *N. locellus* are insect larvae of the genus *Aesha* or freshwater snail *Lymnaea stagnalis*. Comparative analysis of published data shows no essential differences between the morphometrical values of *N. locellus* (=*O. locellus*) larval stages and adult from Bulgarian population, examined by us, and the data for the same parasite species from Central and Southeast Europe reported by Kossak, 1910; Zarnowski, 1960; Genov and Dimitrova, 1966; Genov, 1979, 1984; Prokopič and Genov, 1974; Bock, 1982.
Considerable differences between obtained morphological characteristics and those by Macey and Moore (1958) for *N. locellus* in America have been found. This supported the opinion of Seese (1970), Bocks (1982) and Našincová *et al.* (1989) that the studied by Macey and Moore (1958) trematodes probably belongs to another species of the genus *Neoglyphe*.
The basic chaetotaxy of *N. locellus* cercariae confirm a plagiorchide type of Bayssade-Dufour (1979). Regardless of the use of different methods of impregnation (Combes *et al.*, 1976) and of papillae description (Richard, 1971; Bayssade-Dufour, 1979) with some modifications suggested by Grabda-Kazubska and Kisieline (1989), models of papillae disposition used by Bock (1983) well correspond

Table 1. Measurements (mean ± SD) and classification of the chromosomes of *Neoglyphe locellus*.

Nr	Absolute length $L^a\mu$	Relative length L^r	Centromere index I^c classification
1	4.17	18.26 ± 1.039	48.35 ± 2.333 (m)
2	2.82	12.50 ± 0.205	45.00 ± 7.070 (m)
3	2.72	12.04 ± 0.456	18.50 ± 2.120 (st)
4	2.33	10.22 ± 0.126	38.50 ± 2.120 (m-sm)
5	2.15	9.59 ± 1.019	41.40 ± 1.814 (m)
6	1.61	6.89 ± 0.990	36.66 ± 3.590 (sm-m)
7	1.61	6.89 ± 0.990	36.66 ± 3.590 (sm-m)
8	1.48	6.42 ± 0.326	35.00 ± 7.070 (sm-m)
9	1.43	6.26 ± 0.100	39.00 ± 1.400 (m-sm)
10	1.25	5.62 ± 0.791	39.00 ± 1.400 (m-sm)
11	1.16	5.31 ± 1.238	39.00 ± 1.400 (m-sm)

III. Karyological investigations
The diploid cells of *N. locellus* contain 22 chromosomes with absolute length from 4.17 to 1.16. Mean genome length is 22.43 (Table 1, Fig. 15). The first pair takes 18.26 % from the relative haploid length and differs from the other. The differences in length between 2nd to 11th chromosomes are not statistically significant. According to the

to those in the present study. There were found no significant differences between the sensory apparatus of the populations from Central and South-East Europe which confirm a correct identification of our material. On the grounds of a comparative analysis of the papillae STDL (=C_mD_2 in the present work), A_1D (=$C_{IV}D_1$ and $C_{IV}D_2$) and of ventral papillae on the body (Dimitrov *et al.*, 1989) re-

liable criteria for the differentiation of *O. locellus* from *Opisthioglyphe rastellus* (Olsson, 1876) (according to Richard, 1971), *Opisthioglyphe megastomus* Baer, 1943 (according to Vaucher, 1972), and *Opisthiogyiphe ranae* (Frölich, 1971), according to Dimitrov *et al.* (1989) were found.

Most of the studied genera included into the family Plagiorchiidae have 22 diploid chromosomes (Baršienė, 1993; Mutafova, 1994) and this number can be the modal characteristic for the group. The karyotypes consist mainly of two-armed chromosomes with variation in I^c values in distinct taxons.

The comparative karyological analysis of *N. locellus* with *O. ranae* and *Plagiorchis maculosus* studied by Mutafova (1994) shows a significant differences in the localization of the centromere in some of the corresponding chromosome pairs (Fig. 16). Thus *N. locellus* has only one typical subtelocentric chromosome (No 3), *O. ranae* has 4, (No 3, 4, 5, 6) whereas karyotype of *P. maculosus* consists of two-arm chromosomes with variation of the centromere location in 4 of the chromosome pairs (No 2, 4, 6 and 7). These data show that *N. locellus* and *P. macullosus* have more similar karyotype characteristics than *O. ranae* and it could be suggested that they are phylogenetically closer genera.

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